Detecting latent tuberculosis using interferon gamma release assays (IGRA)

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Tuberculosis
Tip of the Iceberg

Active cases

TB infection

Total Population
Detection of latent tuberculosis

• TB skin test (TST)
  – Intradermal injection of PPD (complex antigen soup that cross-reacts with BCG & some NTMs)
  – After 2-3 days look for induration at injection site
  – Typically performed by nursing staff

• Interferon gamma release assays (IGRA)
  – Blood test performed by laboratory
  – Look for T-cell response to antigens from *M. tb* (production of gamma interferon)
Advantages of blood IGRA

• Advantage in specificity because antigens used are in the RD-1 locus of M. tuberculosis and are not found in the BCG vaccine → BCG vaccinated patients do not test positive

• Is more sensitive than TST in testing immuno-compromised patients

• Only a single clinic visit is required

• Doesn’t create a “booster effect”—risk that repeated injection of PPD could → false +

• Variability of TST readings (but IGRAs have variability issues as well)

• Lab information systems more reliable than TST records
FIG 2 Countries where BCG vaccine is given after infancy or multiple times (at present or in the past). In these settings, IGRAs may be more specific than TST for latent TB infection. (Adapted from reference 12, which was published under a Creative Commons license.)
## Variability of TST readings

<table>
<thead>
<tr>
<th></th>
<th>TST Reader 2 positive</th>
<th>TST Reader 2 negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>TST Reader 1 positive</td>
<td>35</td>
<td>9</td>
</tr>
<tr>
<td>TST Reader 1 negative</td>
<td>7</td>
<td>280</td>
</tr>
</tbody>
</table>

Drawbacks of IGRA

- Can be difficult to validate—there is no “gold standard” for comparison
- Shifts burden of identifying patients with latent TB infection (LTBI) from nursing staff to lab, ↑ demand for lab resources
- T cells can be unstable in blood samples, so fresh blood must be tested
- Variability in repeat testing due to dichotomous cut point & lack of a definition of what is a conversion
  High negative ↔ Low positive
- Calls to lab from M.Ds wanting interpretation, which probably shouldn’t be lab’s role
Performing the T-SPOT.TB test involves the following steps using standard lab equipment:

1. Collect the blood sample. At the lab, PBMCs are separated from whole blood, washed, counted and inoculated into 4 separate microtiter wells.

2. Add WBCs [●] and specific TB antigens [○] to wells pre-coated with antibodies to IFN-γ [↑] and incubate 16 to 20 hours (37°C, CO₂).
QuantiFERON®-TB: 3 generations

- **1st**: QuantiFERON®-TB (liquid antigen)
  - **2001** — FDA-approved
  - Measured the cell-mediated immune response to tuberculin purified protein derivative (PPD) used for the TST

- **2nd**: QuantiFERON-TB Gold (liquid antigen)
  - **2004** — FDA-approved
  - Used antigens specific (ESAT 6 & CFP10) for *M. tuberculosis* complex organisms

- **3rd**: QuantiFERON-TB Gold (*QFT® in tube*)
  - **2007** — FDA-approved
  - Blood collection tubes as incubation vessels
  - Can be fully automated

QFT Procedure

1. Blood draw
2. Shake tubes
3. Incubate
4. Centrifuge
5. Harvest plasma
6. ELISA*
7. Calculate results*

* Typically automated in lab
Precautions for Quantiferon

• Indeterminate results may be caused by
  – Too long an interval between blood collection and testing
  – Insufficient mixing of blood tubes
  – Incomplete washing of the EIA plate

• A negative Quantiferon result does not preclude the possibility of TB infection or disease

• Incorrect performance of the assay may cause a false negative result
Validating an IGRA

• Studies on IGRAs have limitations, namely lack of a gold standard for latent TB infection.

• After plasma is harvested, samples may be shared with a reference laboratory to check accuracy of gamma interferon EIA.

• Check with manufacturer for latest recommendations regarding test validation.

• Results of samples from low-risk and known TB patients may be compared.
IGRAs as a diagnostic tool for active TB

- Sensitivity of IGRAs in active TB is about 75% to 90%
- IGRAs are not as accurate as methods which detect the presence of M. tb bacteria (culture, NAAT, etc.)
- IGRAs can be a useful supplementary test in children who have suspected TB disease (sputum specimen collection is problematic) Lewinsohn Curr Opin Pediatr 2010, 22(1):71-6
Practical considerations, QFT vs. T-Spot

Problem with specimen stability/T cell viability:

- QFT: “In-Tube” method enables any lab with an incubator to perform 1st steps

  • Blood samples were tested fresh and after preservation by new methodology—results very similar, but more data are definitely needed.
    - 2 published studies were supported by the manufacturer
  • Blood samples for T-Spot testing can be overnighted to a national reference lab
Independent evaluation of T-spot XT at Hawaii State Laboratories

<table>
<thead>
<tr>
<th>T.SSPOT result without XT Tested within 4 hours</th>
<th>T.SSPOT result With XT after 24-28 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Positive 27</td>
</tr>
<tr>
<td>Borderline</td>
<td>Positive 2</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive 1</td>
</tr>
<tr>
<td>Total</td>
<td>Total 30</td>
</tr>
</tbody>
</table>

Thanks to Chris Whelen, Lance Chinna, and Matt Bankowski.
Note that a few (4/31) of the patients who were positive when a fresh specimen was tested became borderline after holding the sample 24-48 hours using the XT product. Specimens were held in XT at a stable room temperature. XT did not appear protective, and a few more specimens had lower reactivity.
Boosting of Interferon-gamma Response by TST – QFT-GIT

Van Zyl-Smit et al. AJRCCM 180;2009
CDC recomm, cont’d

**BOTH IGRA and TST** may be recommended if:

- **Initial** test is **negative** and risk of infection or progression to active disease is high, or if signs and symptoms of TB are present
- If the **initial** test is **positive** and additional evidence is required to encourage compliance with preventive therapy, or in healthy persons with low risk of infection and progression
Does a positive IGRA have value in predicting subsequent active TB?

Diel, Loddenkemper, Nienhaus metanalysis

PPV* for IGRAs 2.7%
PPV for TST 1.5%

NPV** for IGRAs 99.7%
NPV for TSTs 99.4%

*PPV = % with a positive result which progressed to active disease
**NPV = % with a negative result who remained disease-free
Can IGRAs be used to monitor the effectiveness of drug treatment for latent TB?

- No
Proportion of health care workers with change from negative to positive results

- Quantiferon Gold In-Tube 6.1%
- T-Spot TB 8.3%
- TST 0.9%

From TBESC Task Order 18, S. Dorman, et al.

Possible flaw in this study: when employees had TST, did this boost the results of follow-up IGRAs?
Some patients:

High negative ↔ low positive

AKA “wobblers”
TB Responses measured by QFT-GIT were **greater:**
Following injection of PPD
When blood volume was less than 0.8 mL (vs. 1.2 mL)
When blood was collected in the evening (6-9 PM vs 6-9 AM)

TB Responses measured by QFT-GIT were **smaller:**
If blood incubation was delayed for 11-12 hrs (vs w/i 1 hr)
If blood incubation was shortened to 16-17 hrs (vs 23 to 24 hrs)
Variability of the enzyme immunoassay component of Quantiferon


Repeated the EIA using the same plasma samples

“...the normal expected range of within-subject variability in TB response on retesting included differences of ±0.60 IU/ml for all individuals and ±0.24 IU/ml for individuals whose initial TB response was between 0.20 and 0.80 IU/ml.”
Mancuso/Mazurek military recruit study

• 2017 military recruits participated
• All had survey about risk factors, TST, T-Spot, QFT, and a “Battey” NTM skin test
• 88 had a positive result: only 10 of these were positive to all 3 tests
• For the majority of positive results, the 3 tests identified different people
• Suggests that in a low prevalence population, most discordant results are due to false positives
IGRA testing of a low risk health care worker population:

- Testing of low-risk populations is not recommended
- If you are requested to perform IGRA testing on a low-risk population, refer to 2016 ATS/CDC/IDSA guidelines
- “…the committee thought that a positive test in a low-risk individual was likely to be a false-positive result, and recommended repeat testing.”
Interpretation of IGRA results

• Interpretation of IGRA results requires consideration of patient history and other clinical information
• Lab staff should not have the role of interpreting IGRA results.
• Lab staff should be aware of limitations of IGRA testing, to assist in planning and policy development
New study evaluating QFT in children
Andrews Lancet Respiratory 2017

• Part of an (unsuccessful) vaccine evaluation in South Africa
• HIV negative children aged 18-24 weeks were enrolled
• 7% of children were QFT positive after ~1 year, with gamma interferon values from 0.35 to >4.
• Of those children with quantitative results 0.35 to 4 did not have a significantly increased risk of TB disease, and 77% reverted to negative QFT
• Of those children with QFT values >4, 28% developed active disease over the next 6-24 months.
• Suggests that IGRAs may be useful in testing children <3, & suggests particular focus on diagnostic and preventive intervention for children with QFT >4.
References


References, cont’d


References, cont’d


  
  Article URL [http://www.occup-med.com/content/7/1/6](http://www.occup-med.com/content/7/1/6)

References, cont’d

